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Supplemental Material

Sex-Specific Effects of Organophosphate Diazinon on the Gut Microbiome and Its Metabolic Functions

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Figure S1. Distinct gut microbiome features were observed in male and female animals, as evidenced by the significant separation on the 3D-PCoA plot (A) (Red: Female; Blue: Male); Hierarchical clustering analysis by UPGMA indicates that male and female mice clustered in a gender-specific manner, with the UPGMA distance tree constructed at a distance of 0.1 (B).

Figure S2. Analysis at the Level 2 of Subsystem illustrate diazinon exposure altered the pathways of Amino acids and derivatives in a gender-selective manner. In male mice, the

pathways of *alanine, serine and glycine* and *aromatic amino acids and derivatives* were statistically significantly perturbed (A). In female mice, the pathway of *branched-chain amino acids* was altered (B). (* indicates p-value <0.05).

Figure S3. Analysis at the Level 3 of Subsystem illustrate diazinon exposure altered the pathways of *aromatic amino acids and derivatives* in a gender-selective manner. In male mice, the specific pathways of *aromatic amino acid degradation* and *Chorismate: intermediate for synthesis of Tryptophan* were statistically significantly perturbed (A). No statistical significance was observed in female mice (B). (* indicates p-value <0.05).

Figure S4. Serum metabolomic profiles were readily differentiated between the controls and diazinon-treated mice using principle component analysis (A: male mice; B: female mice). Pathway analysis using identified altered serum metabolites indicate that 7 and 5 metabolic pathways were significantly perturbed in male (C) and female animals (D) (p value <0.05), respectively. (Each dot represents one metabolic pathway; A metabolic pathway may not reach statistical significance if the number of metabolite hit in that specific pathway is too low; Only pathways with statistical significance are labeled herein).

Figure S5. Molecular features with p value <0.05 and fold change >1.5 were further filtered based on Variable Importance in Projection (VIP) scores associated with partial least squares discriminant analysis (PLS-DA) for the controls and treatment samples. Only features with VIP>1.8 were selected for identification with database search (A: male; B: female). These features are the most important ones to drive the separation between the controls and treatment samples.

Figure S6. The body weight gain was statistically significantly different between the controls and treated male mice after exposure to diazinon for 13 weeks ($p=0.017$ by Welch two sample t-test) (A); However, there was no statistically significant difference between controls and diazinon-treated female animals ($p=0.24$) (B). (All boxes extend from the 25th to the 75th percentile, horizontal bars represent the median, whiskers extend 1.5 times the length of the interquartile range (IQR) above and below the 75th and 25th percentiles, respectively, and outliers are represented as points).